Survival impact of epidermal growth factor receptor overexpression in patients with non-small-cell lung cancer: a meta-analysis

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Key words: epidermal growth factor receptor, meta-analysis, lung cancer, overexpression, survival

Abstract

Background. Overexpression of epidermal growth factor receptor (EGFR) in non-small-cell lung cancer (NSCLC) has been considered to compromise patient survival, presumably by promoting tumor growth by an autocrine mechanism. Yet, considering the conflicting results reported from various laboratories, the issue of clinical importance of EGFR overexpression remains unsettled.

Methods. We carried out a meta-analysis of previous studies to quantitatively review effects of EGFR overexpression on survival in NSCLC patients, using a DerSimonian-Laird random effects model. Eighteen studies including 2972 patients were subjected to final analysis.

Results. Overall, positivity for EGFR overexpression differed between histologic types: 39% in adenocarcinomas, 58% in squamous cell carcinomas, 38% in large-cell carcinomas, and 32% in cancers in a miscellaneous category ($p<0.0001$). The combined hazard ratio (HR) was 1.14 (95% CI, 0.97 to 1.34; $p=0.103$), indicating that EGFR overexpression has no significant survival impact. Considering only the 15 immunohistochemistry-based studies, the combined HR was 1.08 (95% CI, 0.92 to 1.28; $p=0.356$), again suggesting that EGFR overexpression has no survival impact. Heterogeneity testing indicated that heterogeneity between studies existed, but publication bias was absent, suggesting the summary statistics obtained may approximate the actual average.

Conclusions. EGFR overexpression was not associated with poorer survival in patients with NSCLC. Specific mutations of the *EGFR* gene will need further study in terms of survival implications. (215 words)

Introduction

Lung cancer is the deadliest cancer worldwide.[1] Despite tremendous efforts to reduce deaths due to lung cancer, the overall 5-year survival rate is approximately 10% in every country, and has shown little improvement in recent decades.[2] For earlier disease stages of non-small-cell lung cancer (NSCLC), potentially curative therapy is surgical. For more advanced stages, chemotherapy and radiotherapy are regarded as standard treatments. Recently a new strategy, molecular targeted therapy, has been considered as a possible addition to this armamentarium. A prototypical molecular target for clinical application is the epidermal growth factor receptor (EGFR).[3] Activation of EGFR results in enhancement of cell proliferation and suppression of apoptosis.[4][5][6] EGFR therefore seems a logical choice of target for controlling tumor growth.

Among the various anti-EGFR agents, gefitinib[7][8] and erlotinib,[9] both tyrosine kinase inhibitors, have proven useful for patients with advanced NSCLC. In particular, gefitinib is now used as a second- or third-line therapy after platinum-based chemotherapy, obtaining responses in certain tumors showing resistance to other chemotherapy. In other clinical trials, cetuximab, a monoclonal antibody that blocks EGFR, has been confirmed to have antitumor effects in patients with advanced NSCLC.[10] Importance of EGFR as a molecular target thus has been rapidly increasing.

Overexpression of EGFR is thought to imply poor survival, presumably reflecting autocrine stimulation of tumor growth initiated by interaction between EGFR and its ligands, epidermal growth factor (EGF)/transforming growth factor alpha (TGFα), amphiregulin, epiregulin, and beta-cellulin.[11] Association between EGFR overexpression and altered survival in patients with NSCLC has been studied for many

years as a test of this hypotheses. However, conflicting results have been reported from different laboratories. Accurate and conclusive evaluation of the clinical significance of EGFR overexpression is important in order to understand the pharmacologic mechanism of anti-EGFR therapy, and to choose appropriate future molecular targets. We therefore carried out a meta-analysis of published studies to quantitatively review effects of EGFR overexpression on survival of patients with NSCLC.

Materials and Methods

Eligibility criteria for meta-analysis

This meta-analysis was limited to studies dealing with the prognostic implications of EGFR overexpression. The following criteria for eligibility among studies were set before collecting articles. (1) Expression of the *EGFR* gene was evaluated in primary lung cancer tissue as opposed to sera or metastatic tissue. (2) Methods used to evaluate EGFR expression included immunohistochemistry (IHC) or quantification of EGFR mRNA or protein. (3) The histologic type of the tumors was NSCLC. (4) Hazard ratio (HR) and its confidence interval (CI) comparing patients with EGFR overexpression with patients without overexpression were described or statistically extractable from the data in the article. (5) Median follow-up time exceeded 2 years. (6) Articles were published in English in the periodical literature from January 1990 to November 2004. (7) Finally, when multiple articles were published by the same authors or group, the newest or most informative single article was selected.

Collection of published studies

Via the Internet, the MEDLINE data base was searched in November 2004 for bibliographic information concerning articles about EGFR expression status and survival in patients with NSCLC. Key words “lung cancer + epidermal growth factor receptor + prognosis,” “lung cancer + epidermal growth factor + survival,” “lung cancer + HER1,” and “lung cancer + erbB1,“ hit 127, 104, 17, and 20 citations, respectively. Manual selection of relevant studies was carried out based on the summary analysis. Articles found to overlap others or to be unrelated to our question were excluded. When appropriate, items from hand-searched bibliographies were added. Thirty-seven articles concerning association of EGFR expression with survival in NSCLC were found initially. Among these selected articles, HR and its CI were not extractable statistically from 10 articles,[12][13][14][15][16][17][18][19][20][21] which typically reported only p values. Five articles[22][23][24][25][26] originated from investigative groups already represented. In 3 articles[27][28][29] EGFR protein was measured in sera. One article[30] compared a combined EGFR overexpression or underexpression group with a medium-expression group. Accordingly, 19 articles were excluded from the present meta-analysis (Table 1), while 18 studies[31][32][33][34][35][36][37][38][39][40][41][42][43][44] [45][46][47][48] were found to fulfill eligibility criteria (Table 2).

Table 1. Articles excluded from the present meta-analysis.

Author	Year	Reasons for exclusion	Histology: No. of patients	EGFR overexpressin (%)	EGFR effect on survival
Dazzi et al.[12]	1989	No statistical data for extracting HR	Ad:31 Sq:97 La:7 Mis:17	49	NS
Rusch et al.[22]	1993	Updated by Rusch (1997)	Sq:19 Non-sq:25	45	NS
Volm et al.[23]	1993	Updated by Volm (1998)	Total:121	83	S
Giatromanolaki et al.[13]	1996a	No statistical data for extracting HR	Ad:38 Sq:69	79	NS
Giatromanolaki et al.[14]	1996b	No statistical data for extracting HR	Ad:38 Sq:69	79	NS
Pfeiffer et al.[24]	1996	Updated by Pfeiffer (1996)	Ad:59 Sq:102 La:25	55	NS
Koukourakis et al.[15]	1997	No statistical data for extracting HR	Ad:38 Sq:69	ND	NS
Greatens et al.[16]	1998	No statistical data for extracting HR	Ad:51 Sq:44 La:6	65	NS
D'Amico et al.[17]	2000	No statistical data for extracting HR	Ad:208 Sq:148 La:52	52	NS
Cox et al.[25]	2001	Updated by Swinson (2004b)	Ad:50 Sq:105 La:14	56	NS
Volm et al.[18]	2002	No statistical data for extracting HR	Ad:59 Sq:123 La:34	ND	S
Kanematsu et al.[19]	2003	No statistical data for extracting HR	Sq:14 Non-Sq:22	81	NS
Sasaki et al.[27]	2003	Serum protein measured	Ad:51 Sq:42 La:5 Mis:9	34	NS
Brattstrom et al.[20]	2004	No statistical data for extracting HR	Ad:25 Sq:22 La:2 Mis:4	30	NS
Gregore et al.[28]	2004	Serum protein measured	Ad:29 Non-Ad:17	ND	NS
Jacot et al.[29]	2004	Serum protein measured	Ad:56 Sq:127 La:38	ND	NS
Nimeric et al.[30]	2004	Over- and underexpression was compared with medium expression	Sq:78	60	S
Swinson et al.[26]	2004a	Updated by Swinson (2004b)	Ad:49 Sq:107 La:12 Mis:4	ND	NS
Onn et al.[21]	2004	No statistical data for extracting HR	Ad:56 Sq:40 Mis:15	50	NS

EGFR, epidermal growth factor receptor; HR, hazard ratio; Ad, adenocarcinoma; Sq, squamous cell carcinoma; La, large cell carcinoma; Mis, miscellaneous; ND, no data; S, significant; NS, not significant; CFS, cancer-free survival.

Table 2. Studies included in the present meta-analysis.

Author	Year	Method	Specimen	Antibody	Stage	Histology: No. of patients	EGFR overexpression (%)	EGFR effect on survival
Veale et al.[31]	1993	Protein assay (RIA)	Frozen	NA	I-III	Ad:7 Sq:10 La:2	11/19 (58)	S (worse)
Tateishi et al.[32]	1994	IHC	Paraffin	Mouse sera (Transformation Research Inc)	I-IV	Ad:119	55/119 (46)	NS
Pastorino et al.[33]	1997	IHC	Paraffin	Monoclonal (Triton Diagnostics)	I	Ad:217 Sq:252 Mis:46	245/515 (48)	NS
Rusch et al.[34]	1997	IHC	Paraffin	Monoclonal (Triton Diagnostics)	I-IIIA	Ad:52 Sq:36 La:8	68/96 (71)	S (better)
Fontanini et al.[35]	1998	IHC	Paraffin	Monoclonal (Triton Diagnostics)	I-IIIA	Ad:66 Sq:116 Mis:13	92/195 (47)	NS
Pfeiffer et al.[36]	1998	ELISA	Frozen	(Amersham, Oncogene Science)	ND	Ad:57 Sq:100 La:23	49/180 (27)	NS
Volm et al.[37]	1998	IHC	Paraffin	Polyclonal (Dianova)	I-III	Sq:121	100/121 (83)	S (worse)
D'Amico et al.[38]	1999	IHC	Paraffin	Monoclonal (BioGenex Laboratories)	I	Ad:208 Sq:145 Mis:55	212/408 (52)	NS
Fu et al.[39]	1999	IHC	Paraffin	Monoclonal (Sigma)	I-IIIB	Sq:63 Non-Sq:95	104/158 (66)	NS
Ohsaki et al.[40]	2000	IHC	Paraffin	Monoclonal (Novacastra)	I-IV	Ad:142 Sq:127 La:17 Mis:4	124/290 (43)	S (worse)
Brabender et al.[41]	2001	RT-PCR	Frozen	NA	I-IIIA	Ad:32 Sq:39 La:12	28/83 (34)	NS
Piyathilake et al.[42]	2002	IHC	Paraffin	Monoclonal (Zymed Laboratories)	I-IIIA	Sq:60	30/60 (50)*	S (worse)
Hirsch et al.[43]	2003	IHC	Paraffin	Monoclonal (Zymed Laboratories)	I-IIIB	Ad:79 Sq:89 La:15	68/183 (37)	NS
Deeb et al.[44]	2004	IHC	Paraffin	Monoclonal (Dako)	I-IIIA	Ad:83 Sq:43	43/123 (35)	NS
Parra et al.[45]	2004	IHC	Paraffin	Monoclonal (EFeAb-10)	I-IV	Ad:29 Sq:9 Mis:12	23/50** (46)	S (worse)
Selvaggi et al.[46]	2004	IHC	Paraffin	Monoclonal (Oncogene Science)	I-IIIA	Ad:48 Sq:60 La:22	48/130 (37)	S (worse)
Shah et al.[47]	2004	IHC	Paraffin	Monoclonal (Zymed Laboratories)	I-III	Ad:43 Sq:9 Mis:11	48/63 (76)	S (better)
Swinson et al.[48]	2004	IHC	Paraffin	Monoclonal (Novacastra)	I-IIIA	ND	93/179 (52)	NS

EGFR, epidermal growth factor receptor; IHC, immunohistochemistry; ELISA, enzyme-linked immunosorbent assay; RT-PCR, reverse transcription-polymerase chain reaction; Ad, adenocarcinoma; Sq, squamous cell carcinoma; La, large cell carcinoma; Mis, miscellaneous; S, significant; NS, not significant; NA, not applicable; ND, no data; *Median was used for cutoff value. **Treated by gefitinib.

Extraction of hazard ratios

HRs and their 95% CIs were used to combine the data. When described in text or tables, we obtained these values directly from these six articles.[31][33][44][46][47][48] When these statistical variables were not given explicitly in an article, they were calculated from available numerical data in 3 articles[35[38][39] using methods reported by Parmar et al.[49] In summary, HRs and CIs were calculated from difference between numbers of observed and expected events concerning the primary endpoint of length of survival. Published survival curves were used to obtain HR and CI in 9 articles.[32][34][36][37][40][41][42][43][45] In these cases, after dividing the time axis into non-overlapping intervals, log HR and its variance for each interval were calculated. These estimated values were combined in a stratified manner to obtain overall HR and 95% CI.[49]

Statistical analyses

A DerSimonian-Laird random effects analysis[50] was used to estimate the effects of EGFR overexpression on survival. DerSimonian-Laird method is the simplest form of a random effects meta-analysis. To undertake a random effects meta-analysis, the standard errors of the study-specific estimates are adjusted to incorporate a measure of the extent of variation, or heterogeneity, among the treatment effects observed in different studies. The size of this adjustment can be estimated from the treatment effects and standard errors of the studies included in the meta-analysis. This method permits combined assessment of heterogeneous studies to produce combined HR and 95% CI. Heterogeneity among combined studies was tested simultaneously. The statistical software used was StatsDirect, version 2.3.8. Categorical variables such as EGFR

positivity for each histologic type were tested by the Chi-square test. For these analyses, a p value below 0.05 was considered to indicate significance. Evidence of publication bias was sought by the method of Egger et al.[51] and the method of Begg and Mazumdar.[52] For these analyses p values below 0.1 were considered to indicate significance.

Results

A total of 2972 patients were subjected to final analysis (mean, 165 per study; range, 19 to 515). Patients with locoregional disease (stages I to IIIB) were included in 15 studies, while patients in stage IV were included in 3 studies. Surgery was performed for most patients. Only 50 patients in one study [45] were treated with gefitinib. Among the 18 studies included in this meta-analysis, a significant association between EGFR overexpression and survival was demonstrated in 8 studies (44.4%) including 6 (33.3%) linking expression with shorter survival and 2 (11.1%) linking expression with longer survival. The remaining 10 studies (55.6%) yielded negative results.

In the 18 studies included, expression of EGFR was evaluated by IHC in 15, by mRNA expression in 1, by radioimmunoassay (RIA) in 1, and by enzyme-linked immunosorbent assay (ELISA) in 1. Using cutoff values for overexpression chosen by each author, 1441 (48.5%) of the 2972 patients in the meta-analysis had EGFR-positive lung cancers. Only adenocarcinomas were examined in 1 study, and only squamous cell carcinomas were analyzed in 3. The remaining 14 studies investigated all histologic types, although precise numbers of cases for each histologic type were not mentioned in 3 studies.[31][39][48] Positivity for EGFR overexpression in the individual studies ranged

from 27%[36] to 83%.[37] Overall EGFR positivity in the studies included differed according to histologic type: 39% in adenocarcinomas, 58% in squamous cell carcinomas, 38% in large-cell carcinomas, and 32% in miscellaneous lung cancer (Table 3, p<0.0001). Thus, overexpression was significantly more frequent in squamous cell carcinoma.

Table 3. Overall numbers and percentages of EGFR positivity according to histologic type in cases from included studies.

Histologic type	EGFR-positive	p value
Ad	460 / 1175 (39.1%)	<0.0001*
Sq	695 / 1206 (57.6%)	
La	37 / 98 (37.8%)	
Mis	41 / 130 (31.5%)	
Total	1233 / 2609 (45.8%)**	

EGFR, epidermal growth factor receptor; Ad, adenocarcinoma; Sq, squamous cell carcinoma; La, large cell carcinoma; Mis, miscellaneous. *χ^2 test, **Three studies were excluded because they lacked information about EGFR positivity according to histologic type.

Combined HR for all 18 eligible studies was 1.14 (95% CI, 0.97 to 1.34; p=0.103), indicating that EGFR overexpression has no significant survival impact in patients with NSCLC (Fig. 1A). We also separately analyzed studies using only IHC for evaluation. In these 15 IHC studies, the combined HR was 1.08 (95% CI, 0.92 to 1.28; p=0.356), again

suggesting that EGFR overexpression had no significant survival impact (Fig. 1B). Heterogeneity testing revealed that both the group including all studies and the group including only IHC studies were heterogeneous; Q=40.8, p=0.001 for all eligible studies and Q=31.9, p=0.004 for IHC studies. We focus then on the predominant histologic type in each study. When we limited analysis to 5 studies dealing mostly (>50%) with squamous cell carcinomas, the combined HR was 1.40 (95% CI, 0.98 to 2.00; p=0.07) without heterogeneity (Q=8.13, p=0.09; Fig. 1C). When we limited analysis to 6 studies dealing mostly (>50%) with adenocarcinomas, the combined HR was 0.90 (95% CI, 0.63 to 1.28; p=0.55) with inter-study heterogeneity (Q=14.2, p=0.01; Fig. 1D).

Publication bias statistics obtained by the methods of Egger et al.[51] and Begg and Mazumdar[52] were as follows: all 18 eligible studies (p=0.715 and p=0.654), 15 IHC studies (p=0.684 and p=0.697), 5 studies considering mainly squamous cell carcinoma (p=0.153 and p=0.233), and 6 studies considering mainly adenocarcinoma (p=0.525 and p=0.136). This suggested absence of publication bias in all studies.

Discussion

Gene amplification or overexpression of EGFR in lung cancer has been reported since the 1980's.[53][54] Recently EGFR has attracted attention as a molecular target in the treatment of NSCLC. Understanding mechanisms of action of anti-EGFR therapy requires correct evaluation of impact of EGFR expression on patient survival. Combining 18 published studies including 2972 patients with NSCLC yielded summary statistics indicating that EGFR overexpression has no significant survival impact.

Two major types of meta-analysis may be performed, meta-analysis of the literature (MAL) and meta-analysis of individual patient data (MAP).[55] When we can access individual patient data easily, MAP is preferable for more precise statistical evaluation. However, we used MAL, since individual patient data were difficult to access in the 18 different studies published over a 10-year period.

Meta-analysis originally was developed to combine the results of randomized controlled trials.[56] Recently, however, this approach has been applied successfully to identification of prognostic indicators in patients with lung cancer, such as aneuploidy[57] and p53 mutations.[58] We found 1 previous meta-analysis[59] finding an association between EGFR overexpression detected by IHC and poor survival in NSCLC patients. That analysis demonstrated significantly shorter survival based on 8 IHC studies (HR 1.13, 95% CI 1.00 to 1.28). However, in that analysis 1 IHC study[34] showing longer survival in EGFR-overexpressing tumors was excluded, which might have affect the results. Our present analysis of 15 IHC studies suggested that EGFR overexpression has no survival impact.

Heterogeneity testing detected significant heterogeneity between the studies that we analyzed; even when we limited the scope of analysis to IHC studies, heterogeneity still was detected. Since EGFR positivity may be related to histologic type of tumors, we initially thought that heterogeneity may have arisen from the squamous cell carcinomas included. When analysis was limited to the five studies including mostly squamous cell carcinomas, heterogeneity disappeared. On the other hand, when we analyzed only the six studies including mostly adenocarcinomas, heterogeneity still was detected. Accordingly,

histologic type may be neither a major cause of heterogeneity nor a decisive factor concerning the biologic effect of EGFR overexpression.

Factors related to immunostaining also can cause heterogeneity. Atkins et al.[60] studied variables affecting results of immunostaining for EGFR in paraffin-embedded specimens, concluding that fixation solution and prolonged storage of archival tissue section might affect positivity of cells. Other differences concerning staining techniques and evaluation criteria for EGFR positivity might result in heterogeneity between studies.

Publication bias[61] is a well known problem in meta-analysis; positive results tend to be accepted by journals, while negative results often are rejected or not even submitted.[62] However, our analysis did not suggest publication bias, so the summary statistics obtained may approximate the actual average.

In our meta-analysis, when HR and CI were not described in the reports, they were extracted from the data according to Parmar et al.[49] We should keep in mind that estimated HR values and their CIs may differ to some extent depending on the estimation method used.

Theoretically, gefitinib might most effective for squamous cell carcinomas, which express ample EGFR in more than half of cases. However, data obtained so far[63][64] suggest that most effective response may be observed in adenocarcinomas. This discrepancy may require considerable examination to be resolved. In recent studies,[65][66][67] adenocarcinomas that showed missense substitutions or small in-frame deletions within the coding region of the ATP-binding pocket in the *EGFR* gene responded well to gefitinib therapy. The mutant EGFRs may selectively activate Akt and signal transduction and activator of transcription (STAT), which strongly suppress

apoptosis.[5] Gefitinib induces apoptosis by preventing mutant EGFR from activating these pathways.

In contrast to the impression above, Kim et al.[68] reported that overexpression of EGFR in adenocarcinoma did not predict response to gefitinib. Further, Perez-Soler et al.[69] reported that EGFR overexpression was not a significant predictor for either tumor response to erlotinib or survival after such treatment. In combination, these results and our own suggest that EGFR mutations rather than overexpression are likely to be important determinants of the biologic behavior of NSCLC.

In conclusion, EGFR overexpression was not associated with survival in patients with NSCLC in the present meta-analysis. Although overexpression frequently was detected in squamous cell carcinoma, survival impact was unaffected when studies were selected according to the percentages of squamous cell carcinoma or adenocarcinoma. Since somatic mutations within the *EGFR* gene are known to result in inhibition of apoptosis, mutations of this gene will require further study in terms of survival implications for NSCLC patients.

Acknowledgements

This study was in part funded by Grant No. 15591493 from the Ministry of Education, Science, Sports, and Culture of Japan.

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Figure legend

Figure 1. Meta-analysis of hazard ratio (HR) of the effect of epidermal growth factor receptor (EGFR) overexpression on survival in patients with non-small-cell lung cancer (NSCLC). Bars indicate 95% confidence interval (CI) of HR in patients with EGFR overexpression. Areas of squares are proportional to weights used in combining data. The center of the lozenge gives the combined HR. Effect of EGFR on survival was considered statistically significant if the 95% CI for combined HR did not overlap the value 1. A: All 18 eligible studies. B: 15 immunohistochemically evaluated studies. C: 5 studies including over 50% squamous cell carcinoma cases. D: 6 studies including over 50% adenocarcinoma cases.

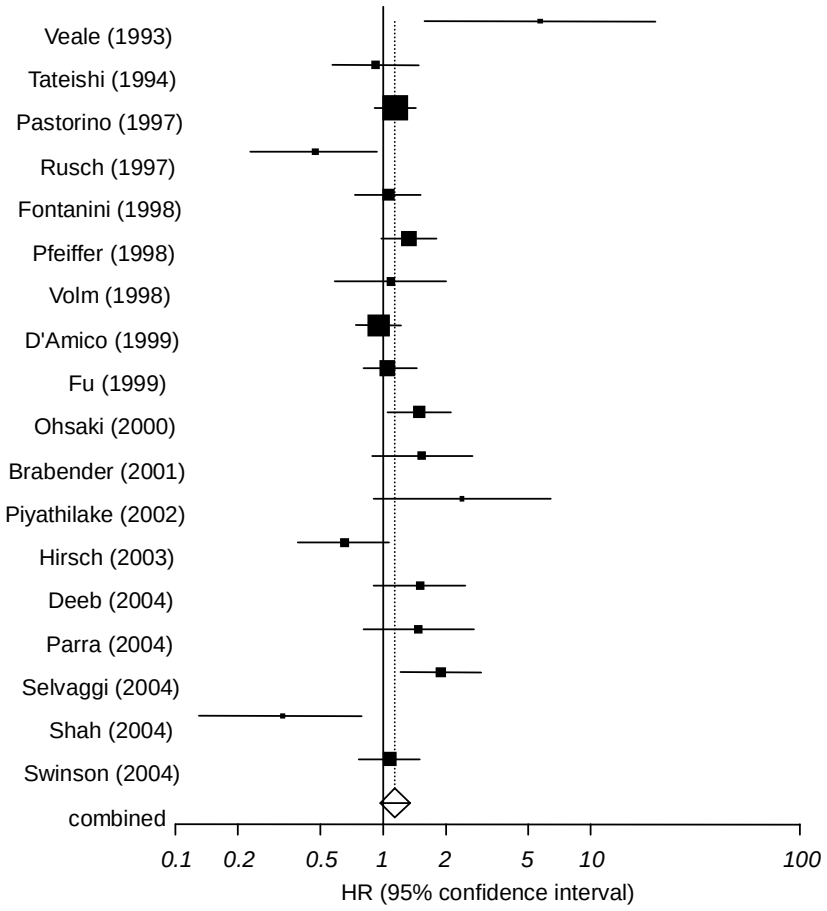
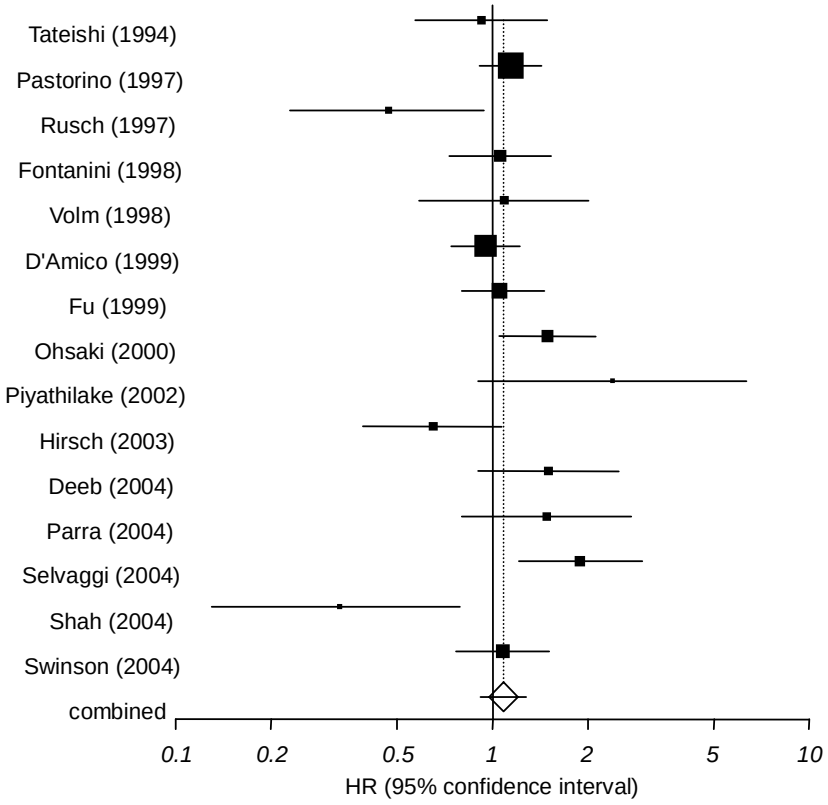


Fig. 1A



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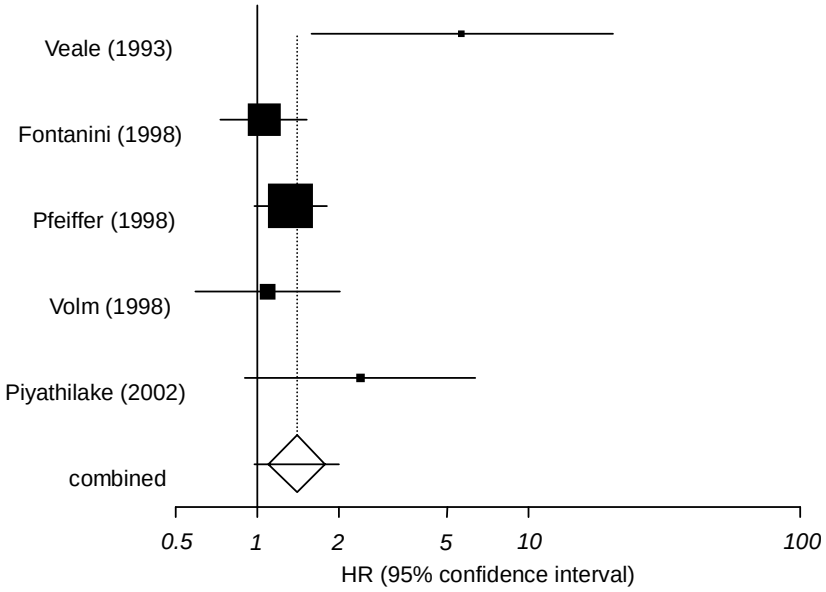

Fig. 1C

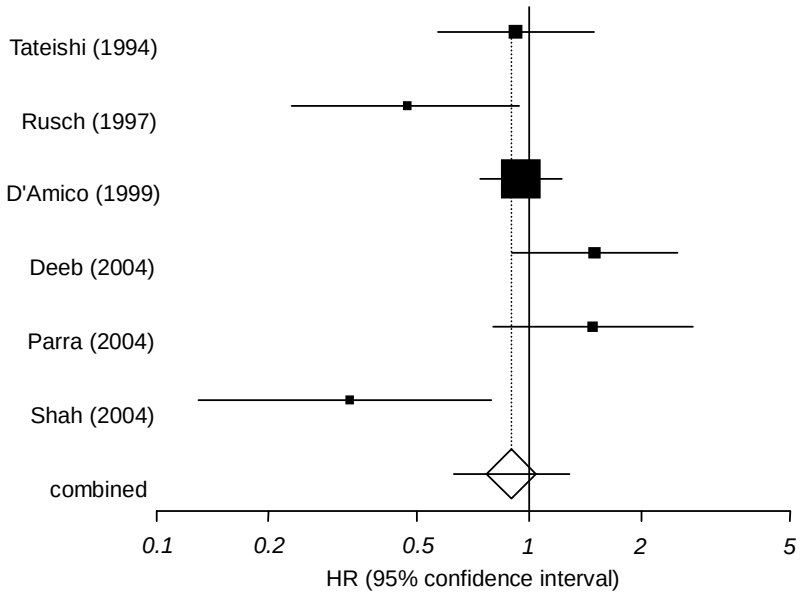

Fig. 1D